Milk production responses, rumen fermentation, and blood metabolites of dairy cows fed increasing concentrations of forage rape (*Brassica napus* ssp. *biennis*)

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ABSTRACT

The aim of this study was to determine animal performance, rumen fermentation, and health-related blood metabolites of dairy cows in mid lactation fed with increasing levels (30 and 45%) of forage rape (FR) in the diet. Twelve pregnant multiparous lactating Holstein-Friesian dairy cows were randomly allocated to 1 of 3 dietary treatments in a replicated 3 × 3 Latin square design. The experiment was divided into three 21-d periods. For the control diet, 13.0 kg (dry matter, DM) of grass silage, 3.0 kg DM of commercial concentrate, 2.7 kg of DM cold-pressed extracted canola meal, and 0.45 kg DM of solvent-extracted soybean meal were offered daily. For the other two treatments, 30 and 45% of the DM from silage, canola meal, and commercial concentrate were replaced in equal proportions with FR. Data were analyzed individually using linear and quadratic orthogonal polynomials. Ingestive behavior was altered by the inclusion of FR. We observed a linear increase in eating time at the expense of rumination time. Nevertheless, total DM intake was not affected by dietary treatments, averaging 19.5 ± 0.24 kg of DM/d. Milk yield increased linearly with increasing concentration of FR in the diet. Thus, feed efficiency of cows (kg of milk/kg of DM intake) increased linearly with the percentage of FR in the diet. Inclusion of FR in the diet had no effect on milk composition or milk sensory characteristics. Mean rumen pH of cows decreased linearly from the control to the 45% FR diet; however, dietary treatments had no effect on the daily amount of time that rumen pH was below 5.8 (252 ±

71.4), indicating no risk of subacute ruminal acidosis. Concentrations of total volatile fatty acids in the rumen and molar proportions of acetate and butyrate were increased with FR inclusion, whereas the proportion of propionate was linearly reduced. Excretion of uric acid and total purine derivatives tended to be greater for cows fed FR, which resulted in a trend toward a linear increase in estimated microbial N flow. However, N use efficiency was not affected by FR inclusion. Although differences for some hematological measures (increased white blood cell and neutrophils counts) and a quadratic response for glutamate dehydrogenase for cows fed FR in the diet (decreased with inclusion of 30% and increased with 45% in the diet) were observed, all values were within appropriate ranges for dairy cows. These results indicated that including FR to dairy cow diets, up to 45% of diet DM, improved milk production due to changes in volatile fatty acids and predicted microbial N flow and had no negative effects on dairy cow health or sensory characteristics of milk.

Key words: *Brassica*, volatile fatty acids, purine derivatives, health

INTRODUCTION

Forage rape (FR; *Brassica napus* ssp. *biennis*) is a crop used in grazing systems to supply supplementary feed in times of low pasture growth because it (1) can produce high yields of DM (8–15 t of DM/ha) in a relatively short period (60–120 d; Barry, 2013); (2) has low establishment costs; (3) uses water and nitrogen efficiently; and (4) has high concentrations of digestible DM and CP (Kaur et al., 2011). This crop can be incorporated in an annual crop rotation to improve feeding budgets in pasture-based systems (Garcia et al., 2008). In terms of nutrient concentration, FR is highly digestible (75–85% digestible OM), contains between 10 and 20% CP, has low NDF (18–20%) and high con-

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centrations of readily fermentable carbohydrates such as starch (6–11%), sugars (10–15%), and pectins (9%), and therefore ferments rapidly in the rumen (Sun et al., 2012; Westwood and Mulcock, 2012; Keim et al., 2019).

Inclusion of FR in the diet can improve animal feed conversion efficiency compared with legumes (Kaur et al., 2010) or silage plus concentrate diets (Castillo-Umaña et al., 2020). This reduces feeding costs and increases farm profitability because homegrown forages are frequently less expensive than purchased concentrates (Garcia et al., 2008). Moreover, FR has been shown to reduce the environmental impact of sheep grazing pasture diets (Sun et al., 2012) and dairy cows fed alfalfa hay or chicory (Williams et al., 2016) through lower CH₄ production either per day or per kilogram of ECM.

However, FR and other brassica forages contain secondary compounds [e.g., S-methyl-cysteine sulfoxide (SMCO), glucosinolates, and nitrates] in greater concentrations than other forages that can cause nutritional disorders and subclinical alterations that are implicated in reduced voluntary intake and underperformance, affect rumen fermentation, and alter sensory characteristics of milk (Barry, 2013). Other characteristics of FR, such as high moisture concentration, inadequate fiber concentration, and altered rumen fermentation, may also contribute to negative effects on animal performance or health when these crops are fed (Lambert et al., 1987). Cattle allocated high rates (ad libitum) of FR and preferentially consuming FR leaves may be at greater risk of SARA, because of the low NDF content and high concentrations of readily fermentable carbohydrates (Westwood and Mulcock, 2012).

Thus, it has been recommended that brassicas can be provided as a minor proportion (0.2–0.3) of dairy cow diets to dilute secondary compounds (Westwood and Mulcock, 2012; Barry, 2013). Castillo-Umaña et al. (2020) observed that although DMI was reduced with inclusion of 25% FR in the diet, milk production was not affected. Conversely, Williams et al. (2016) reported no decrease in DMI and greater milk production with dairy cows fed 43% FR in an alfalfa hay-based diet. However, health condition and milk characteristics were not reported in their study.

The aim of this study was to determine DMI, rumen fermentation, health-related blood metabolites, milk sensory characteristics, and production responses of mid-lactation dairy cows fed increasing concentrations (30 and 45%) of FR in the diet. We hypothesize that FR can be included in the diet of mid-lactation dairy cows without negatively affecting DMI, rumen fermentation, health-related blood metabolites, milk sensory characteristics, or production responses.

MATERIALS AND METHODS

The study was conducted at the Experimental Research Station of Universidad Austral de Chile, Valdivia, Chile, between January and March 2018. All experimental procedures were approved by the Universidad Austral Institutional Animal Care and Use Committee (Approval Number: 237/2015).

Animals, Housing, and Experimental Design

Twelve pregnant multiparous lactating Holstein-Friesian dairy cows (24.1 ± 0.72 kg of milk/d, 540 ± 7.3 kg of BW, and 142 ± 3.9 DIM at the start of the study; $\pm$ SD) were used in this study. All animals were housed in the same tiestall barn, equipped with stalls with rubber bedding. Animals were milked twice daily at 0700 and 1600 h in the milking parlor close to the barn facility and had ad libitum access to water.

Cows were randomly allocated to 1 of 3 dietary treatments in a replicated 3×3 Latin square design. The experiment lasted 63 d and was divided into three 21-d periods. The first 14 d of each period was for adaptation of the animals to the diets, and the last 7 d was used for data collection. Seven days before the first experimental period, cows were housed for adaptation to the experimental conditions and offered the control diet, which was composed of 13.0 kg DM of grass silage, 3.0 kg DM of commercial concentrate, 2.7 kg DM of cold-pressed extracted canola meal, and 0.45 kg DM of soybean meal (Table 1). The amount offered daily was increased proportionally for each feed ingredient to achieve ~5% orts.

Brassica Crop Production and Animal Feeding

The ‘Spitfire’ cultivar of FR was sown in October and November 2017 in 2 adjacent 0.5-ha areas at a density of 4 kg of seed/ha. The crop was sown on 2 dates at a 20-d interval so that we could offer plant material at a similar stage of maturity throughout the experiment. Daily, FR was harvested with a cutter bar mower (Bertolini 140L, Reggio Emilia, Italy) at 10 cm above ground level and transported to the animals.

Cows were divided into 3 dietary treatments: (1) control, (2) 30% FR, and (3) 45% FR (Table 1). The control diet was composed of 67% grass silage, 15.5% commercial concentrate, 2.3% soybean meal, 13.9% canola meal, and 1.3% mineral salts of total DM offered. For the other 2 dietary treatments, 30 and 45% of the DM from silage (46.2 and 35.7% of total DM offered for 30% FR and 45% FR), commercial concentrate (10.7 and 8.2% of total DM offered for 30% FR and 45% FR), and cold-pressed extracted canola meal

Table 1. Ingredient and chemical composition of the experimental diets (% of DM basis unless stated)

Item	Diet ¹		
	Control	30% FR	45% FR
Ingredient			
Grass silage ²	67.0	46.2	35.7
Commercial concentrate ³	15.5	10.7	8.2
Soybean meal ⁴	2.3	2.3	2.3
Canola meal ⁵	13.9	9.6	7.4
Forage rape ⁶	0	30.0	45.0
Mineral and vitamin supplement	1.3	1.3	1.3
Chemical composition			
DM	56.6	42.5	36.4
Ash	6.9	9.0	10.1
aNDFom ⁷	38.7	32.4	29.2
ADFom ⁷	24.2	20.8	19.1
CP	17.0	17.4	17.6
Lipid	4.9	4.3	3.9
NFC ⁸	29.1	33.6	35.7
Digestible OM	74.4	75.5	75.9
NE _L (Mcal/kg DM)	1.71	1.73	1.74

¹30% FR = 70% control diet and 30% forage rape; 45% FR = 55% control diet and 45% forage rape.

²Grass silage composition: 41% DM, 8% ash, 48.8% NDF, 12.4% CP, 3.2% lipids, 72% dOM (OM degraded, as a % of original OM), NE_L: 1.65 Mcal/kg of DM.

³Commercial concentrate composition: 87% DM, 3.4% ash, 22.8% NDF, 17.3% CP, 6.1% lipids, 87% dOM, NE_L: 1.99 Mcal/kg of DM.

⁴Soybean meal composition: 88% DM, 7.4% ash, 7.8% NDF, 52.3% CP, 1.2% lipids, 91% dOM, NE_L: 2.09 Mcal/kg of DM.

⁵Canola meal composition: 90% DM, 6.1% ash, 21.5% NDF, 34.2% CP, 12.9% lipids, 78% dOM, NE_L: 1.79 Mcal/kg of DM.

⁶Forage rape composition: 11.5% DM, 14.1% ash, 18.8% NDF, 19.9% CP, 2.9% lipids, 78% dOM, NE_L: 1.79 Mcal/kg of DM.

⁷aNDFom = NDF in the OM; ADFom = ADF in OM, determined by sequential fiber analysis with correction for residual ash (Cassida et al., 2007).

⁸Calculated according to NRC (2001): NFC = 100 – (% NDF + % CP + % fat + % ash).

(9.6 and 7.4% of total DM offered for 30% FR and 45% FR) were replaced with FR ($46.3 \pm 3.2\%$ leaf, $23.0 \pm 1.9\%$ petioles, and $30.7 \pm 4.9\%$ stems). The amount of soybean meal remained constant to keep the 3 diets isoenergetic and isonitrogenous, which met the NE_L and MP requirements of the cows based on NRC (2001) recommendations.

Before feeding, all feeds were weighed and offered individually to each cow according to the dietary treatments. Grass silage and canola meal were offered twice a day at 0800 and 1700 h and soybean meal was offered once a day at 1700 h along with mineral salts. Once cows consumed the canola and soybean meal, grass silage was offered. For FR treatments, orts of grass silage were removed at 1100 and 1900 h, and a fresh portion of FR was offered at the same allowance for a.m. and p.m. feeding. Commercial concentrate was offered twice daily during milkings (0700 and 1600 h).

Intake and Ingestive Behavior

The amount of feeds offered and orts were weighed for each cow and recorded on d 1, 3, and 5 of wk 3 of

each period. Subsamples of each feed ingredient and orts were collected and DM content was determined by weighing before and after drying in a forced-air oven at 105°C for 12 h and then stored for further nutrient concentration analysis. Nutrient intake of each feed ingredient was calculated as the difference between the amount offered multiplied by its nutrient concentration, and the dry weight of orts multiplied by its nutrient concentration. In case of FR and grass silage, there were always some orts, whereas supplements were completely consumed. Samples of forages (grass silage and FR) were freeze-dried and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA) before chemical analyses. Leaves + petioles and stems of FR were analyzed separately. Samples for chemical analyses of commercial concentrate, soybean meal, and canola meal were taken once per experimental period. For each sample, ash and lipids were analyzed according to AOAC International (1996; method 942.05 and 920.39 for ash and ether extract, respectively); N content was determined by combustion (model FP-428 Nitrogen Determinator, Leco Corp., St Joseph, MI) and was used to calculate CP ($N \times 6.25$). Neutral

detergent fiber was determined as NDF in the OM (aNDFom; Van Soest et al., 1991) using sodium sulfite (Merck, Darmstadt, Germany) and heat-stable amylase (Ankom Technology Corp., Macedon, NY), and ADF in the OM (ADFom) according to AOAC International (1996; method 973.18). Sequential fiber analysis with correction for residual ash was conducted, as pectins and ash can interfere with detergent fiber analysis of brassica forages (Cassida et al., 2007); OM degraded as a percent of original DM was measured according to Tilley and Terry (1963), and NE_L was estimated according to NRC (2001).

Behavior was evaluated based on the following activities: eating (bite procurement, chewing between bites, or searching), rumination (standing or lying behavior of the animal), and other activities (resting, social interactions, drinking, demonstration of estrus, and others). Each activity was visually and continuously recorded by trained operators on d 4 of each experimental week, with each operator working in sequences of 6 h. Every 10 min, from 0800 to 1600 h and from 1700 to 0700 h (presence of the cows in the barn), operators noted the activity that each cow was performing. Each activity's time was calculated by summation of all 10-min intervals of activity.

Production, Composition, and Sensory Characteristics of Milk

Cows were milked at 0700 and 1600 h and milk yield was recorded daily with a flow sensor (MPC580 DeLaval, Tumba, Sweden) during the experimental periods. The daily average for the final week of each period is reported. Representative milk samples were collected with Waikato MK V lactometers (Waikato, New Zealand) at the morning and afternoon milkings for 3 d in the last week of the experimental period to determine fat, protein, lactose, and MUN analyses by mid-infrared spectrophotometry (Foss 4300 Milko-Scan, Foss Electric, Hillerød, Denmark).

For descriptive sensory analysis, milk samples of cows under the same treatment were pooled within a period. Milk samples were pasteurized (65°C for 25 min) and presented to the sensory panel at 20°C. The panel was composed of 10 trained judges who were not provided any information about the previous treatment of the milk samples. The judges were given 25 mL of milk in coded plastic glasses and evaluated the following attributes: milk odor, general flavor, spiciness, and bitterness. The judges evaluated the milk samples sequentially by rating the attributes on a continuous intensity scale from 1 to 9, where 1 represents a highly inadequate attribute and 9 highly adequate (Seguel et al., 2020). Scores from each judge were considered an

analytical replicate, and the average value for pooled milk samples within an experimental period was considered the experimental unit.

Rumen Fermentation

Rumen fluid was harvested by stomach tubing (Flora Rumen Scoop; Prof-Products, Guelph, ON, Canada) after each milking at 0800 and 1700 h on d 6 of wk 3 in each experimental period. Samples were strained through 4 layers of cheesecloth. A 10-mL sample was drawn off, mixed with 0.2 mL of 50% (wt/vol) sulfuric acid, and stored at -20°C pending determination of VFA and NH_3 concentrations. Rumen fluid was allowed to thaw for 16 h at 4°C and then centrifuged at $10,000 \times g$ for 10 min at 4°C . Six milliliters of supernatant was drawn off and centrifuged at $10,000 \times g$ for 10 min at 4°C . Thawed supernatant of rumen fluid samples was analyzed for VFA by GC as described by Tavendale et al. (2005) and for NH_3 by the phenol-hypochlorite reaction method (Weatherburn, 1967). Minor VFA are the sum of isobutyrate, isovalerate, valerate and caproate.

For the whole experiment, rumen pH was monitored by wireless telemetric pH bolus (eCow, Exeter, UK). The pH boluses were calibrated before use, programmed to measure rumen pH at 15-min intervals, and inserted directly into the ventral sac of the rumen of each cow. The data were transmitted wirelessly to a transceiver connected to a cellphone and then transferred to a laptop computer. The pH data were summarized as mean pH, pH per hour, and time spent at $\text{pH} > 6.2$, $5.8 \leq \text{pH} \leq 6.2$, and $\text{pH} < 5.8$.

Urine Collection and Purine Derivative Measurements

To predict rumen microbial N (MN) flow based on purine derivatives (PD), spot urine samples (20 mL) were collected by subvulvar stimulation every 3 h during d 5 of wk 3 in each experimental period. Samples were acidified with 2 mL of H_2SO_4 (10% vol/vol) to maintain pH below 3 and stored at -20°C . Urine samples were thawed and a composite sample per cow was made for each period and analyzed for allantoin, uric acid, and creatinine by HPLC. The equations used in calculating the predicted MN outlined below have been described previously (Totty et al., 2013).

The PD index was calculated based on total PD [allantoin (mmol/L) + uric acid (mmol/L)]:

$$\text{PD index} = \{[\text{total PD (mmol/L)}] / \text{creatinine (mmol/L)}\} \times \text{BW}^{0.75},$$

where $BW^{0.75}$ is metabolic BW.

Urine volume was estimated using creatinine concentration as a marker and assuming a daily creatinine excretion of 26 mg/kg of BW (Lindberg, 1989). The estimated urinary creatinine excretion ($0.9 \text{ mmol/kg of } BW^{0.75}$) was included in the following equation to estimate the daily excretion of PD ($\text{mmol/kg of } BW^{0.75}$):

$$\text{daily excretion of PD (dPD; mmol/kg of } BW^{0.75}) = \text{PD index} \times 0.9.$$

From this, the amount of purines absorbed daily was estimated:

$$\text{daily absorbed purines (daP)} = [\text{dPD (mmol/kg of } BW^{0.75}) - 0.385 \times BW^{0.75}] + 0.85.$$

Finally, MN (g/d) was estimated using the following equation:

$$\text{MN (g/d)} = (\text{daP} \times 70) / (0.116 \times 0.83 \times 1,000).$$

Hematological and Biochemical Blood Measures

Blood samples were collected after morning milking on d 4 of wk 3 in each period. Blood was collected from a coccygeal vessel using 2 evacuated blood collection tubes (BD Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ): 1 containing EDTA (4 mL) and 1 with no anticoagulant (9 mL). Samples were transported on ice to the hematology laboratory of the Universidad Austral de Chile Teaching Hospital for analysis, where they were centrifuged at $3,000 \times g$ for 10 min at 25°C . Whole blood was used for determination of red blood cell (RBC) counts, hemoglobin (Hb) concentration, hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), white blood cell (WBC) counts, and differential leucocytic count, using an automated hematology analyzer (KX-21N, Sysmex, Kobe, Japan) was used. Heinz bodies were counted in blood smears prepared on average 5 h after sampling, using crystal violet staining, and expressed as percent erythrocytes containing one or more inclusion bodies. Serum samples were stored at -20°C until analyzed for BUN, γ -glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), nonesterified fatty acids (NEFA), BHB, and aspartate aminotransferase (AST), using a Wiener Metrolab 2300 auto-analyzer (Wiener Lab, Rosario, Argentina) at 37°C . Thyroxine (T_4) and triiodothyronine (T_3) were measured using a validated Elisa Kit (MyBioSource, San Diego, CA).

Statistical Analyses

Data were analyzed using the mixed model procedure of SAS (SAS Institute Inc., Cary, NC) to account for carryover effects according to the following model:

$$y_{ijklm} = \mu + S_i + A_{(i)j} + P_{(i)k} + T_l + C_m + e_{(ijk)l},$$

where y_{ijklm} is an observation for each dependent variables; μ is the general mean; S_i is the fixed effect of the i th treatment sequence ($i = 1$ to 6); $A_{(i)j}$ is the random effect of the j th cow in the i th sequence; $P_{(i)k}$ is the fixed effect of the k th period ($k = 1$ to 3); T_l is the fixed effect of the l th treatment ($l = 1$ to 3); C_m is the fixed carryover effect from the previous period ($C = 0$, if period = 1); and $e_{(ijk)l}$ is the random error. If carryover effects were not detected, a simplified model for a replicated Latin square was used:

$$y_{ijkl} = \mu + S_i + A_{(i)j} + P_{(i)k} + T_l + e_{(ijk)l},$$

where y_{ijkl} is the observation for dependent variables; μ is the general mean; S_i is the random effect of the i th square ($i = 1$ to 4); $A_{(i)j}$ is the random effect of cow nested within square; $P_{(i)k}$ is the fixed effect of the k th period ($k = 1$ to 3); T_l is the fixed effect of the l th treatment ($l = 1$ to 3); and $e_{(ijk)l}$ is the random error. Data for DM and nutrient intake, behavior, milk yield, milk composition, PD, MN, time of pH under a threshold value, and hematological and biochemical blood measures were summarized by day. For hematological measures, 2 tubes were not reported by the laboratory due to coagulation of samples; therefore, different standard errors are reported for each treatment. Sampling time (a.m. or p.m. sampling for VFA and NH_3) and hour (for pH) were included as repeated measurements and the interaction of treatment and repeated measurement, with cow as a subject. In case of pH and NH_3 , the interaction between dietary treatment and time was not significant, and therefore it was removed from the model. The estimation method was REML and the degrees of freedom method was Kenward-Roger. The variance-covariance structure that yielded the lowest corrected Akaike information criterion was compound symmetry, which was selected for the final model. For sensory analysis of milk variables, a completely randomized block design was used, where block corresponded to the experimental period. Orthogonal polynomial contrasts were performed to determine linear and quadratic effects of the inclusion of FR. The IML procedure of SAS (SAS Institute Inc.) was used to generate coefficients adjusted for the unequal spacing of increasing FR inclusion in the diet. All data were

reported as LSM $\pm$ SEM. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Intake, Ingestive Behavior, and Production, Composition, and Sensory Characteristics of Milk

Dry matter, feed and nutrient intakes, ingestive behavior, and milk production are presented in Table 2. Grass silage intake was reduced linearly by 4.7 and 6.1 kg of DM for cows fed 30% FR and 45% FR compared with the control diet ($P < 0.001$) respectively, whereas FR intake increased linearly by 6.0 and 8.4 kg of DM from the control diet to 30% FR and 45% FR ($P < 0.001$). Nevertheless, inclusion of FR in the diet had no effect on total daily DMI or digestible OM intake,

which averaged 19.5 ± 0.2 and 14.7 ± 0.2 kg/d, respectively. Whereas CP intake increased linearly ($P < 0.001$) with the inclusion of FR in the diet, a linear decline was observed for OM, aNDFom, and ADFom intakes ($P < 0.01$). Ingestive behavior was altered by the inclusion of FR in the diet; the time that cows spent eating increased linearly from 252 to 418 min/d ($P < 0.001$), whereas rumination time reduced linearly from 539 to 338 min/d when including FR up to 45% in the diet ($P < 0.001$). The time that cows spent performing other activities showed a quadratic response (649, 634, and 685 min/d for control, 30% FR, and 45% FR, respectively; $P = 0.036$).

We detected a linear effect of including FR in the diet on milk ($P = 0.042$) and lactose yield ($P = 0.043$), and a trend for a linear response for ECM ($P = 0.069$), fat- and protein-corrected milk ($P = 0.099$), and protein

Table 2. Feed and nutrient intake, ingestive behavior, milk yield and milk composition of cows fed with increasing concentrations of forage rape in their diet

Variable	Diet ¹			SEM	P-value	
	Control	30% FR	45% FR		Linear	Quad ²
Feed intake (kg of DM/d)						
Grass silage	13.5	8.8	7.4	0.15	<0.001	0.018
Forage rape	0	6.0	8.4	0.14	<0.001	0.261
Total	19.6	19.6	19.4	0.24	0.531	0.705
Nutrient intake (kg/d)						
OM	18.0	17.5	17.2	0.20	0.008	0.723
CP	3.2	3.5	3.6	0.05	<0.001	0.309
aNDFom ³	7.8	6.4	5.9	0.08	<0.001	0.071
ADFom ⁴	4.9	4.1	3.9	0.05	<0.001	0.067
dOM ⁵	14.6	14.8	14.7	0.18	0.607	0.486
Ingestive behavior (min)						
Eating	252	376	418	12.4	<0.001	0.388
Ruminating	539	430	338	10.5	<0.001	0.068
Other activities	649	634	685	13.9	0.168	0.036
Milk yield (kg/d)	23.4	25.2	24.9	0.06	0.042	0.258
ECM ⁶ (kg/d)	24.3	25.9	25.9	0.80	0.069	0.471
FPCM ⁷ (kg/d)	24.0	25.4	25.5	0.92	0.099	0.223
Milk composition (%)						
Fat	3.88	3.80	3.88	0.06	0.878	0.385
CP	3.12	3.13	3.13	0.01	0.856	0.845
Lactose	4.89	4.86	4.87	0.02	0.459	0.504
Milk component yields (kg/d)						
Fat	0.90	0.95	0.96	0.02	0.141	0.779
CP	0.73	0.79	0.78	0.01	0.051	0.265
Lactose	1.14	1.22	1.21	0.04	0.043	0.292
Feed efficiency ⁸						
kg of milk/kg of DMI	1.20	1.29	1.28	0.04	0.022	0.253
kg of ECM/kg of DMI	1.25	1.34	1.33	0.04	0.054	0.118
kg of FPCM/kg of DMI	1.23	1.31	1.32	0.05	0.087	0.618

¹30% FR = 70% control diet and 30% forage rape; 45% FR = 55% control diet and 45% forage rape.

²Quad = quadratic effects of orthogonal polynomial contrasts.

³aNDFom = NDF in the OM.

⁴ADFom = ADF in the OM, determined by sequential fiber analysis with correction for residual ash (Cassida et al., 2007).

⁵dOM = OM degraded as a % of original OM.

⁶ECM = (12.97 $\times$ kg of fat) + (7.21 $\times$ kg of protein) + (0.3273 $\times$ kg of milk); Tyrrell and Reid (1965).

⁷Fat- and protein-corrected milk = milk (kg/d) $\times$ (0.1226 $\times$ fat % + 0.0776 $\times$ protein % + 0.2534); IDF (2015).

⁸Efficiencies calculated as milk (kg/d), ECM (kg/d), or FPCM (kg/d) divided by DMI (kg/d).

yield ($P = 0.051$). Concentrations of fat, protein, and lactose in milk, and milk fat yield were not modified by dietary treatments, averaging 3.85 ± 0.06 , 3.13 ± 0.01 , $4.87 \pm 0.02\%$, and 0.94 ± 0.02 kg/d, respectively ($P > 0.05$). Feed efficiency (kg of milk/kg of DMI) increased linearly ($P = 0.022$) with inclusion of FR in the diet, whereas trends toward a linear increase for kilograms of ECM/kilogram of DMI ($P = 0.054$) and kilograms of fat- and protein-corrected milk/kilogram of DMI ($P = 0.087$) were observed. Milk sensory analyses showed that inclusion of FR in lactating dairy cows diet had no effect ($P > 0.05$) on general flavor, smell, spiciness, or bitterness of milk, with average scores of 6.73 ± 0.10 , 6.89 ± 0.15 , 6.97 ± 0.12 , and 7.99 ± 0.12 , respectively (Supplemental Table S1; <https://doi.org/10.3168/jds.2020-18785>).

Rumen Fermentation, Urinary PD, and N Metabolism

Results of rumen fermentation measurements are presented in Table 3. There were significant dietary treatment by sampling time interactions for total VFA and molar percentages of propionate, butyrate, isobutyrate, valerate, caproate, and minor VFA ($P < 0.05$). A linear effect of FR inclusion was observed on molar percentages of acetate ($P = 0.069$), isovalerate ($P = 0.069$), and acetate:propionate (A:P) and (acetate + butyrate):propionate [(A+B):P] ratios ($P < 0.001$). Total VFA concentrations in the rumen were not affected by dietary treatments in the morning but increased in the afternoon with the inclusion of FR (88, 96, and 100 mM for control, 30% FR, and 45% FR respectively; $P = 0.011$). The inclusion of FR in the diet increased

linearly the molar percentage of acetate from 62.2 to 64.1 mol/100 mol ($P = 0.002$). In the morning, the molar percentage of propionate in the rumen was linearly reduced (from 20.9 mol/100 mol for cows fed control to 17.9 mol/100 mol for 45% FR); a linear reduction was also observed in the afternoon, but to a lesser extent (16.9 to 15.0 mol/100 mol for cows fed control and 45% FR, respectively; $P < 0.001$). In contrast, butyrate molar percentage in the rumen increased linearly ($P < 0.001$) in the morning (from 14.4 to 16.5 mol/100 mol) and in the afternoon but to a lesser extent than in the morning (13.9 to 14.5 mol/100 mol). These resulted in linear increases in A:P (3.93, 4.26, and 4.54 for control, 30% FR, and 45% FR, respectively) and (A+B):P ratios (4.76, 5.19, and 5.52 for control, 30% FR, and 45% FR, respectively). Molar percentages of minor VFA (4.38 to 4.73 mol/100 mmol), isobutyrate (0.95 to 1.01 mol/100 mol), and caproate (0.45 to 0.62 mol/100 mol) increased linearly with inclusion of FR in the morning; however, no treatment effect was observed in the afternoon. For valerate, there was a quadratic response in the morning (1.46, 1.66, and 1.67 mol/100 mol for control, 30% FR, and 45% FR, respectively) and a linear increase in the afternoon sampling (1.16, 1.23, and 1.30 mol/100 mol for control, 30% FR, and 45% FR, respectively). Finally, a linear reduction was observed with inclusion of FR on rumen isovalerate molar percentage (1.21, 1.13, and 1.12 for control, 30% FR, and 45% FR, respectively; $P = 0.002$).

Mean rumen pH decreased linearly from 6.10 in the control diet to 6.07 in 45% FR ($P < 0.001$; Table 4). The daily times with rumen pH < 5.8 and > 6.2 were not affected by dietary treatments ($P > 0.05$), whereas

Table 3. Effect of increasing concentrations of forage rape on total VFA and in the molar proportions of individual VFA in the rumen fluid of lactating dairy cows

Variable	Diet ¹						SEM	P-value	
	0800 h			1700 h				Linear	Quad ²
	Control	30% FR	45% FR	Control	30% FR	45% FR			
Total VFA (mM)	104	101	105	88	96	100	3.26	0.011	0.401
VFA (mol/100 mol)									
Acetate (A)	60.3	60.5	60.9	64.1	66.6	67.3	0.41	0.002	0.301
Propionate (P)	20.9	19.0	17.9	16.9	15.8	15.0	0.35	<0.001	0.634
Butyrate (B)	14.4	16.0	16.5	13.9	14.5	14.5	1.48	<0.001	0.130
Isobutyrate	0.95	0.97	1.01	0.79	0.75	0.76	0.02	<0.001	0.001
Valerate	1.46	1.66	1.67	1.16	1.23	1.30	0.03	<0.001	0.394
Isovalerate	1.52	1.44	1.44	0.91	0.82	0.81	0.06	0.002	0.435
Caproate	0.45	0.53	0.62	0.29	0.30	0.31	0.17	<0.001	0.349
Minor VFA ³	4.38	4.60	4.73	3.15	3.10	3.18	0.08	0.002	0.412
A:P	2.89	3.22	3.44	3.93	4.26	4.54	0.09	<0.001	0.352
(A+B):P	3.58	4.07	4.37	4.76	5.19	5.52	0.11	<0.001	0.436

¹30% FR = 70% control diet and 30% forage rape; 45% FR = 55% control diet and 45% forage rape.

²Quad = quadratic effects of orthogonal polynomial contrasts.

³Minor VFA are the sum of isobutyrate, isovalerate, valerate, and caproate.

Table 4. Effect of increasing concentrations of forage rape in the diet on rumen pH, urinary purine derivative (PD) and N metabolism of lactating dairy cows

Variable	Diet ¹			SEM	P-value	
	Control	30% FR	45% FR		Linear	Quad ²
Mean rumen pH	6.10	6.06	6.07	0.04	<0.001	0.162
pH <5.8 (min/d)	249	284	225	71.4	0.856	0.458
pH >5.8 and <6.2 (min/d)	667	843	820	46.0	0.029	0.238
pH >6.2 (min/d)	535	453	457	70.1	0.497	0.523
Ammonia (mmol/L)	8.7	10.1	10.9	0.70	0.015	0.902
Allantoin (mmol/d)	418	430	423	5.06	0.301	0.272
Uric acid (mmol/d)	52	68	63	5.15	0.053	0.169
Creatinine (mmol/d)	130	134	131	1.51	0.339	0.202
Total PD excretion (mmol/d)	470	498	486	8.36	0.081	0.156
Predicted microbial N ³ (g/d)	308	326	319	5.12	0.058	0.153
MUN (mg/dL)	12.5	12.7	13.0	0.12	0.027	0.734
BUN (mg/dL)	14.2	14.4	16.6	0.61	0.001	0.010
Milk N (g/d)	114	123	123	2.8	0.026	0.381
Excreted N (g/d)	435	467	475	12.1	0.014	0.647
N use efficiency (%)	20.9	21.1	20.7	0.6	0.857	0.835

¹30% FR = 70% control diet and 30% forage rape; 45% FR = 55% control diet and 45% forage rape.

²Quad = quadratic effects of orthogonal polynomial contrasts.

³Predicted assuming daily purine derivative excretion (dPD; mmol/kg of BW^{0.75}) = PD index × 0.9; daily absorbed purines (daP) = [dPD (mmol/kg of BW^{0.75}) - 0.385 × BW^{0.75}] + 0.85; and microbial N (g of N/d) = (daP × 70)/(0.116 × 0.83 × 1,000).

the daily time with pH between 5.8 and 6.2 had a linear increase of 153 min/d from control to 45% FR ($P = 0.029$).

Results of urinary PD and N metabolism are presented in Table 4. Rumen NH₃ concentration had a linear increase ($P = 0.015$) from cows fed the control diet (8.7 mmol/L) to those fed 45% FR (10.9 mmol/L). Although dietary treatments had no effect on allantoin and creatinine daily excretions, uric acid ($P = 0.053$) and total PD excretions ($P = 0.081$) tended to increase linearly, which resulted in a trend toward a linear increase in predicted MN ($P = 0.058$) with the inclusion of FR in the diet. Concentrations of BUN ($P = 0.001$) and MUN ($P = 0.027$) increased linearly from 14.2 to 16.6 mg/dL and from 12.5 to 13.0 mg/dL, respectively. Milk ($P = 0.026$) and excreted N ($P = 0.014$) from cows showed a linear increase up to 9 and 40 g/d, respectively, with the inclusion of 45% of FR. The N use efficiency of dairy cows was not affected by the inclusion of FR, averaging $20.9 \pm 0.6\%$ among treatments.

Hematological and Biochemical Blood Measures

Hematological measurements such as RBC, Hb, PCV, MCV, MCHC, eosinophils, and lymphocytes were not affected by dietary treatments, whereas neutrophils and leukocytes increased linearly with inclusion of FR in the diet ($P < 0.001$), and a trend for a linear increase was observed for monocytes ($P = 0.082$; Table 5). Among liver enzymes, GGT activity was not affected by dietary treatments, AST activity tended to decrease linearly ($P = 0.069$), and GLDH concentrations showed

a quadratic response ($P = 0.029$), with 57.1, 32.9, and 49.6 U/L for control, 30% FR, and 45% FR, respectively. Lipid mobilization metabolites (NEFA and BHB) and thyroid function (T₃ and T₄) were not affected by dietary treatments ($P > 0.05$). Furthermore, no Heinz bodies were detected in blood from cows fed any of the dietary treatments.

DISCUSSION

The aim of this study was to determine DMI, rumen fermentation, health status, milk sensory characteristics, and production responses of mid-lactating dairy cows fed diets containing increasing percentages (30 and 45%) of FR in the diet. Including FR up to 45% in the diet of dairy cows improved milk performance without causing negative effects on dairy cow health status or on milk sensory properties. It is worth noting that observations of feeding behavior and sampling of urine, blood, and rumen fluid were performed once per experimental period; thus, daily variation in these measurements may have not been captured.

DMI and Ingestive Behavior

Total DMI was not affected by treatments, which indicates that inclusion of FR up to 45% of the diet did not limit intake. The lack of effect on total DMI could be associated with the longer time spent eating by cows offered FR in the diet. As total eating time increased with FR inclusion, ruminating time was reduced. Williams et al. (2016) also reported similar DMI between

cows fed with and without FR. However, a reduction in the DMI of dairy cows has been reported when FR was included at 25% of the diet (Castillo-Umaña et al., 2020). Thus, other factors such as secondary metabolites, water content, and type of diet could influence the effect of FR on DMI (Barry, 2013). For example, the high water content and bulkiness of brassicas increase the fresh weight that animals have to ingest, which may affect eating time (Stefanski et al., 2010). As in our study, Castillo-Umaña et al. (2020) reported an increase in eating time and a reduction in rumination time. This increase in eating time may also be partly due to cows sorting and choosing leaves over stems (Stefanski et al., 2010). The lower DMI for cows fed FR diets reported by those authors may differ from our results (no reduction in DMI with FR inclusion) because of the lower time that cows had access to FR (5 vs. 17 h) and because FR was offered in 1 meal, whereas in the current study it was offered in 2 meals. Behavioral adaptations of cows may be insufficient to maintain daily intake when time at pasture is restricted to less than 8 h (Pérez-Ramírez et al., 2008). The reduction in rumination time may also be explained as a behavioral adaptation of cows to increased eating time. Moreover, it might be affected by the lower aNDFom intake of cows fed FR, because

lower fiber intakes are usually associated with lower rumination time (Zebeli et al., 2012).

Production, Composition, and Sensory Characteristics of Milk

Although DMI was not affected by FR inclusion, milk production increased linearly with the inclusion of up to 45% FR in the diet. The linear increase in feed efficiency (kg of milk/kg of DMI) for cows fed FR may be related to the greater intake of readily fermentable carbohydrates, the increase in total VFA concentration in the rumen, and the tendency to a greater MN flow. Castillo-Umaña et al. (2020) and our study both show an increase in feed efficiency with the inclusion of FR. These indications of greater efficiency suggest that replacing grass silage and purchased concentrates with FR may reduce feeding costs and farm profitability might therefore be increased, because the cost per kilogram DM of purchased concentrates is about 2.5 times that of brassicas (Eckard et al., 2001).

Williams et al. (2016) reported that the inclusion of FR in the diet increased milk production in lactating dairy cows in response to a greater ME intake. In the present study, partial replacement of grass silage with

Table 5. Effect of increasing concentrations of forage rape in the diet on hematological and biochemical health metabolites of lactating dairy cows

Metabolite ¹	Diet ²			P-value	
	Control	30% FR	45% FR	Linear	Quad ³
Hematological					
RBC ($\times 10^6/\mu\text{L}$)	4.8 $\pm$ 0.2	5.4 $\pm$ 0.3	4.9 $\pm$ 0.2	0.547	0.928
Hemoglobin (g/dL)	8.1 $\pm$ 0.2	8.3 $\pm$ 0.2	8.1 $\pm$ 0.22	0.907	0.348
PCV (%)	24.8 $\pm$ 0.7	26.0 $\pm$ 0.8	25.0 $\pm$ 0.7	0.673	0.243
MCV (fL)	53.8 $\pm$ 1.9	53.4 $\pm$ 2.0	53.3 $\pm$ 1.9	0.762	0.968
MCHC (g/L)	327 $\pm$ 6.1	319 $\pm$ 6.7	322 $\pm$ 6.1	0.517	0.593
WBC ($\times 10^3/\mu\text{L}$)	6.7 $\pm$ 0.3	7.4 $\pm$ 0.3	7.8 $\pm$ 0.3	<0.001	0.687
Eosinophils (cells/ μL)	457 $\pm$ 74	460 $\pm$ 79	420 $\pm$ 74	0.693	0.727
Neutrophils (cells/ μL)	2,606 $\pm$ 335	3,308 $\pm$ 392	3,911 $\pm$ 324	<0.001	0.575
Lymphocytes (cells/ μL)	3,335 $\pm$ 261	3,306 $\pm$ 278	3,009 $\pm$ 261	0.272	0.451
Monocytes (cells/ μL)	367 $\pm$ 67	489 $\pm$ 74	539 $\pm$ 67	0.082	0.210
Biochemical					
NEFA (mmol/L)	0.14 $\pm$ 0.02	0.15 $\pm$ 0.02	0.15 $\pm$ 0.02	0.853	0.924
BHB (mmol/L)	0.55 $\pm$ 0.05	0.508 $\pm$ 0.05	0.570 $\pm$ 0.05	0.926	0.251
GGT (U/L)	100 $\pm$ 25	76 $\pm$ 225	90 $\pm$ 26	0.526	0.371
AST (U/L)	124 $\pm$ 14	82 $\pm$ 8	92 $\pm$ 14	0.069	0.245
GLDH (U/L)	57.1 $\pm$ 11.7	32.9 $\pm$ 11.7	49.6 $\pm$ 11.9	0.220	0.029
T ₃ (nmol/L)	0.989 $\pm$ 0.09	0.851 $\pm$ 0.09	0.938 $\pm$ 0.09	0.320	0.158
T ₄ (nmol/L)	106.7 $\pm$ 12.8	116.3 $\pm$ 12.3	116.0 $\pm$ 12.5	0.225	0.633
Heinz bodies (%)	ND ⁴	ND	ND	—	—

¹PCV = hematocrit; RBC = red blood cell count; WBC = white blood cell count; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin volume; NEFA = nonesterified fatty acids; GGT = γ -glutamyl transpeptidase; AST = aspartate aminotransferase; GLDH = glutamate dehydrogenase; T₃ = tri-iodothyronine; T₄ = thyroxine.

²30% FR = 70% control diet and 30% forage rape; 45% FR = 55% control diet and 45% forage rape.

³Quad = quadratic effects of orthogonal polynomial contrasts.

⁴ND = not detected.

FR increased NFC concentration in the diet. Considering that the NFC present in FR are rapidly fermentable (>50% of sugar, starch, and soluble fiber that degrade at rates of 16 to 20%/h; Keim et al., 2019), these may have led to the greater total VFA concentration in the rumen of cows fed FR (Getachew et al., 2004). Moreover, inclusion of FR in the diet consistently increases butyrate molar percentage (Williams et al., 2016; Castillo-Umaña et al., 2020). According to Seymour et al. (2005), the greater energy intake of cows is channeled largely through increases in rumen production of butyrate and propionate and their yield of ATP to the host animal. Finally, the tendency to greater MN flow suggests a greater supply of MP and may explain differences in milk yield, as MN is the major contributor to MP (Schwab and Broderick, 2017). Increasing MP supply up to 9.7% of DMI has been shown to increase milk production (Wang et al., 2007).

The lack of changes in milk composition with FR inclusion in the diet is in accordance with Williams et al. (2016) and Castillo-Umaña et al. (2020) under alfalfa hay and grass silage-based diets, respectively. Evaluation of the sensory characteristics of milk products has been the ultimate method for evaluating product quality (Schiano et al., 2017). It has been proposed that inclusion of brassicas in the diet may affect milk sensory characteristics, such as flavor and odor, because thiocyanate from brassicas has been found to pass into milk and result in flavor defects (Wiedenhoeft and Barton, 1995). Thiocyanates are quickly absorbed and passed into milk; therefore, it is recommended to remove brassicas from cows 4 h before milking to reduce this negative effect (Moate et al., 1996). Moreover, inclusion of FR has been found to modify the fatty acids profile of milk (Williams et al., 2016; Seguel et al., 2020), which can result in oxidized flavors in fluid milk (Schiano et al., 2017). In the present study, the judging panel was unable to detect differences in the sensory characteristics of milk (flavor, smell, spiciness, and bitterness) following inclusion of FR in dairy cow diets. This agrees with previous research reporting that cheeses made of milk from brassica-fed cows were similar in flavor and texture to those made from milk of cows without brassicas in their diet, and no bitter flavors or taint were detected (Moate et al., 1996; Seguel et al., 2020).

Rumen Fermentation, Urinary PD, and N Metabolism

After feeding FR, a linear increase in total VFA was observed, which, as stated earlier, is related to the greater concentrations of readily fermentable carbohydrates in FR compared with grass silage. According to Keim et al. (2019), FR contains between 100 and 130 g/kg of sugars and >300 g/kg DM of soluble fiber,

with 90 g/kg DM of pectins (Sun et al., 2012). Greater inclusion of sugars in lactating dairy cow diets results in greater rumen butyrate concentrations (Oba, 2011), whereas soluble fiber is expected to increase acetate in the rumen (Zhao et al., 2013). This would explain the greater proportions of acetate and butyrate obtained in the rumen of FR-fed cows. Furthermore, fermentable starch increases propionate concentration in the rumen (Oba et al., 2015); thus, the lower propionate concentration in the rumen may be due to the lower starch concentrations in FR diets, in which the inclusion of grain-based concentrate was reduced. The linear increase in minor VFA with the inclusion of FR may be attributed to the greater CP intake by cows with FR in their diets. In the rumen, minor VFA are produced via deamination and decarboxylation processes of the branched-chain AA from feed or microbial protein (Liu et al., 2018).

Dairy cows that are allocated to high allowances of FR preferentially consume leaves over stems (Stefanski et al., 2010) and may be at greater risk of SARA because of the low NDF and high readily fermentable carbohydrate concentrations of FR. Thus, it has been recommended to include brassicas as a minor proportion (0.2–0.3) of the dairy cow diet to dilute secondary compounds in the diet and to prevent SARA (Westwood and Mulcock, 2012; Barry, 2013). Although intake of NDF was lower for cows fed FR, this had no effect on the daily amount of time with pH below 5.8. Although sugars and soluble fiber are readily and extensively broken down in the rumen, they do not mimic the pH-lowering effect of starch and do not produce lactate and rumen pH reduction as starch does (Hall et al., 1998; Oba et al., 2015). Moreover, it has been proposed that the bulkiness of brassicas may increase saliva production, which buffers changes in rumen pH (Keogh et al., 2009). The time that rumen pH was below 5.8 was averaged 250 min/d for all treatments; thus, FR inclusion up to 45% in the diet is not expected to induce SARA, which is defined as a daily decrease in pH <5.8 from 284 to 475 min/d (AlZahal et al., 2007).

Although diets in this study were formulated to be isonitrogenous, the greater N intake may have been due to selection by dairy cows for leaves over stems of FR (Stefanski et al., 2010), as the CP concentration is ~5 percentage units greater in FR leaves than in stems (Keim et al., 2020). This CP degrades rapidly (0.34/h) and to a high extent (85% effective degradability, calculated with a 0.06 passage rate; Keim et al., 2019), increasing the supply of degradable protein in the rumen that produces NH₃. Ammonia is toxic to rumen microorganisms and to the host animal; therefore, it is transported from the rumen to the liver to be converted into urea (Abdoun et al., 2006; Pacheco and

Waghorn, 2008). Furthermore, the trend for a linear increase in MN flow suggests a greater supply of AA in the small intestine. Amino acids that are surplus to requirements will be catabolized and contribute to the urea pool (Lapierre et al., 2005). In contrast to our results, Castillo-Umaña et al. (2020) observed that inclusion of 25% FR in the diet had no effect on N intake and reduced rumen NH_3 concentrations, which might be explained by the greater CP concentrations in grass silage and lower CP concentrations in FR in their study. The literature shows that CP concentration of FR is highly variable and ranges between 10.8 and 27.6% of DM (Keim et al., 2019). As observed in a previous study, MN flow tends to increase with FR (Castillo-Umaña et al., 2020), which might be due to the greater energy supply in the rumen from the fast fermentation rates of their carbohydrates (Keim et al., 2019) or to changes in microbial communities (Sun et al., 2015). These effects resulted in greater retention of N in milk. However, this was accompanied by greater N excretion and no improvement in N use efficiency, as greater N intakes may increase both excreted and milk N (Kebreab et al., 2002).

Hematological and Biochemical Blood Measurements

Forage rape contains SMCO and glucosinolates, which can produce negative effects in animals (Chorfi et al., 2015). Fermentation of SMCO in the rumen produces dimethyl disulfide, which can inactivate Hb and induce anemia (Barry, 2013). Indicators of anemia (hematocrit, Hb, and RBC counts) were similar between cows fed with and without FR. Also, the absence of Heinz bodies suggests that inclusion of FR up to 45% was not associated with short-term negative effects from SMCO. Glucosinolates are fermented in the rumen into isothiocyanates, which can affect function of thyroid hormones (Barry, 2013). However, the concentrations of T_3 and T_4 were not modified by inclusion of FR in the diet. Similar results were reported by Castillo-Umaña et al. (2020), where inclusion of FR at 25% of the diet was not associated with anemia or altered thyroid function. Neutrophil count is a simple measurement to assess the inflammatory status of an animal (Moretti et al., 2015). In this study, WBC counts showed a greater increase, driven by higher neutrophil counts in cows fed FR; however, these values were within the normal range for adult cows ($4.4\text{--}10.8$ and $0.8\text{--}5.0 \times 10^3/\mu\text{L}$ for WBC and neutrophils, respectively; Herman et al., 2018). Thus, the increased leukocytes and especially neutrophils in FR-fed cows may not be associated with inflammatory alterations. Liver health was evaluated according to enzymes released in response to liver cell

damage; that is, AST, GLDH, and GGT (Bionaz et al., 2007). Even though AST and GLDH tended to be reduced with the inclusion of FR in the diet, the values in all treatments were within the reference limits indicated by Noro et al. (2013; <110 and 30 U/L for AST and GLDH, respectively). Moreover, the similar concentrations of GGT suggest that in this study, liver cells were not affected by inclusion of FR, as previously reported by Cox-Ganser et al. (1994).

CONCLUSIONS

The inclusion of FR in dairy cow diets modified nutrient intake and feeding behavior and improved production efficiency and ruminal fermentation, possibly associated with the greater supply of rumen fermentable carbohydrates and microbial protein synthesis. Moreover, no negative effects associated with the health status of cows or sensory characteristics of milk were observed with inclusion of up to 45%. We conclude that, under similar feeding conditions, FR can be included up to 45% in the diet and has the potential to improve milk production efficiency without negative effects on animal health or milk characteristics of mid-lactation dairy cows.

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REFERENCES

- Abdoun, K., F. Stumpff, and H. Martens. 2006. Ammonia and urea transport across the rumen epithelium: A review. *Anim. Health Res. Rev.* 7:43–59. <https://doi.org/10.1017/S1466252307001156>.
- AlZahal, O., E. Kebreab, J. France, and B. W. McBride. 2007. A mathematical approach to predicting biological values from ruminal pH measurements. *J. Dairy Sci.* 90:3777–3785. <https://doi.org/10.3168/jds.2006-534>.
- AOAC International. 1996. *Official Methods of Analysis*. 16th ed. AOAC International, Washington, DC.
- Barry, T. N. 2013. The feeding value of forage brassica plants for grazing ruminant livestock. *Anim. Feed Sci. Technol.* 181:15–25. <https://doi.org/10.1016/j.anifeedsci.2013.01.012>.
- Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Berton. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. *J. Dairy Sci.* 90:1740–1750. <https://doi.org/10.3168/jds.2006-445>.
- Cassida, K. A., K. E. Turner, J. G. Foster, and O. B. Hesterman. 2007. Comparison of detergent fiber analysis methods for forages high in pectin. *Anim. Feed Sci. Technol.* 135:283–295. <https://doi.org/10.1016/j.anifeedsci.2006.07.004>.
- Castillo-Umaña, M. A., O. Balocchi, R. Pulido, P. Sepulveda-Varas, D. Pacheco, S. Muetzel, R. Berthiaume, and J. P. Keim. 2020. Milk production responses and rumen fermentation of dairy cows supplemented with summer brassicas. *Animal* 14:1684–1692. <https://doi.org/10.1017/S175173112000021X>.

- Chorfi, Y., Y. Couture, G. F. Tremblay, R. Berthiaume, and D. Cinq-Mars. 2015. Growth and blood parameters of weaned crossbred beef calves fed forage kale (*Brassica oleracea* ssp. *acephala*). *Adv. Agric.* 2015:1–7. <https://doi.org/10.1155/2015/410497>.
- Cox-Ganser, J. M., G. A. Jung, R. T. Pushkin, and R. L. Reid. 1994. Evaluation of brassicas in grazing systems for sheep. 2. Blood composition and nutrient status. *J. Anim. Sci.* 72:1832–1841. <https://doi.org/10.2527/1994.7271832x>.
- Eckard, R. J., A. A. Salardini, M. Hannah, and D. R. Franks. 2001. The yield, quality and irrigation response of summer forage crops suitable for a dairy pasture renovation program in north-western Tasmania. *Aust. J. Exp. Agric.* 41:37–44. <https://doi.org/10.1071/EA00053>.
- Garcia, S. C., W. J. Fulkerson, and S. U. Brookes. 2008. Dry matter production, nutritive value and efficiency of nutrient utilization of a complementary forage rotation compared to a grass pasture system. *Grass Forage Sci.* 63:284–300. <https://doi.org/10.1111/j.1365-2494.2008.00636.x>.
- Getachew, G., P. H. Robinson, E. J. DePeters, and S. J. Taylor. 2004. Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. *Anim. Feed Sci. Technol.* 111:57–71. [https://doi.org/10.1016/S0377-8401\(03\)00217-7](https://doi.org/10.1016/S0377-8401(03)00217-7).
- Hall, A., A. N. Pell, and L. E. Chase. 1998. Characteristics of neutral detergent-soluble fiber fermentation by mixed ruminal microbes. *Anim. Feed Sci. Technol.* 70:23–39. [https://doi.org/10.1016/S0377-8401\(97\)00068-0](https://doi.org/10.1016/S0377-8401(97)00068-0).
- Herman, N., C. Trumel, A. Geffré, J. Braun, M. Thibault, F. Schelcher, and N. Bourges-Abella. 2018. Hematology reference intervals for adult cows in France using the Sysmex XT-2000iV analyzer. *J. Vet. Diagn. Invest.* 30:678–687. <https://doi.org/10.1177/1040638718790310>.
- IDF (International Dairy Federation). 2015. A common carbon footprint approach for dairy: The IDF guide to standard lifecycle assessment methodology for the dairy sector. Bulletin no. 445. IDF, Brussels, Belgium.
- Kaur, R., S. C. Garcia, W. J. Fulkerson, and I. Barchia. 2010. Utilisation of forage rape (*Brassica napus*) and Persian clover (*Trifolium resupinatum*) diets by sheep: Effects on whole tract digestibility and rumen parameters. *Anim. Prod. Sci.* 50:59–67. <https://doi.org/10.1071/EA08309>.
- Kaur, R., S. C. Garcia, W. J. Fulkerson, and I. M. Barchia. 2011. Degradation kinetics of leaves, petioles and stems of forage rape (*Brassica napus*) as affected by maturity. *Anim. Feed Sci. Technol.* 168:165–178. <https://doi.org/10.1016/j.anifeedsci.2011.04.093>.
- Kebreab, E., J. France, J. A. N. Mills, R. Allison, and J. Dijkstra. 2002. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. *J. Anim. Sci.* 80:248–259. <https://doi.org/10.2527/2002.801248x>.
- Keim, J. P., J. Cabanilla, O. A. Balocchi, R. G. Pulido, and A. Bertrand. 2019. *In vitro* fermentation and *in situ* rumen degradation kinetics of summer forage brassica plants. *Anim. Prod. Sci.* 59:1271–1280. <https://doi.org/10.1071/AN17534>.
- Keim, J. P., M. Gandarillas, D. Benavides, J. Cabanilla, R. G. Pulido, O. A. Balocchi, and A. Bertrand. 2020. Nutrient concentrations and profile of non-structural carbohydrates vary among different *Brassica* forages. *Anim. Prod. Sci.* <https://doi.org/10.1071/AN19472>.
- Keogh, B., P. French, J. J. Murphy, J. F. Mee, T. McGrath, T. Storey, J. Grant, and F. J. Mulligan. 2009. A note on the effect of dietary proportions of kale (*Brassica oleracea*) and grass silage on rumen pH and volatile fatty acid concentrations in dry dairy cows. *Livest. Sci.* 126:302–305. <https://doi.org/10.1016/j.livsci.2009.06.010>.
- Lambert, M. G., S. M. Abrams, H. W. Harpster, and G. A. Jung. 1987. Effect of hay substitution on intake and digestibility of forage rape (*Brassica napus*) fed to lambs. *J. Anim. Sci.* 65:1639–1646. <https://doi.org/10.2527/jas1987.6561639x>.
- Lapierre, H., R. Berthiaume, G. Raggio, M. C. Thivierge, L. Doepel, D. Pacheco, P. Dubreuil, and G. E. Lobley. 2005. The route of absorbed nitrogen into milk protein. *Anim. Sci.* 80:11–22. <https://doi.org/10.1079/ASC41330011>.
- Lindberg, J. E. 1989. Nitrogen-metabolism and urinary-excretion of purines in goat kids. *Br. J. Nutr.* 61:309–321. <https://doi.org/10.1079/BJN19890119>.
- Liu, Q., C. Wang, G. Guo, W. J. Huo, S. L. Zhang, C. X. Pei, Y. L. Zhang, and H. Wang. 2018. Effects of branched-chain volatile fatty acids on lactation performance and mRNA expression of genes related to fatty acid synthesis in mammary gland of dairy cows. *Animal* 12:2071–2079. <https://doi.org/10.1017/S1751731118000113>.
- Moate, P. J., D. E. Dalley, C. Grainger, A. Goudy, T. Clarke, P. Williams, and G. Limsowtin. 1996. The effect of feeding turnips on the concentration of thiocyanate in milk and consequences for cheese making. *Aust. J. Dairy Technol.* 51:1–5.
- Moretti, P., M. Probo, N. Morandi, E. Trevisi, A. Ferrari, A. Minuti, M. Venturini, S. Paltrinieri, and A. Giordano. 2015. Early post-partum hematological changes in Holstein dairy cows with retained placenta. *Anim. Reprod. Sci.* 152:17–25. <https://doi.org/10.1016/j.anireprosci.2014.11.019>.
- Noro, M., P. T. Cid, C. F. Wagemann, V. V. Arnés, and F. M. Wittwer. 2013. Valoración diagnóstica de enzimas hepáticas en perfiles bioquímicos sanguíneos de vacas lecheras. *Revi. Mvz Córdoba* 18:3474–3479. <https://doi.org/10.21897/rmvz.170>.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. The National Academies Press, Washington, DC.
- Oba, M. 2011. Review: Effects of feeding sugars on productivity of lactating dairy cows. *Can. J. Anim. Sci.* 91:37–46. <https://doi.org/10.4141/CJAS10069>.
- Oba, M., J. L. Mewis, and Z. Zhining. 2015. Effects of ruminal doses of sucrose, lactose, and corn starch on ruminal fermentation and expression of genes in ruminal epithelial cells. *J. Dairy Sci.* 98:586–594. <https://doi.org/10.3168/jds.2014-8697>.
- Pacheco, D., and G. C. Waghorn. 2008. Dietary nitrogen—Definitions, digestion, excretion and consequences of excess for grazing ruminants. *Proc. N. Z. Grassl. Assoc.* 70:107–116.
- Pérez-Ramírez, E., R. Delagarde, and L. Delaby. 2008. Herbage intake and behavioural adaptation of grazing dairy cows by restricting time at pasture under two feeding regimes. *Animal* 2:1384–1392. <https://doi.org/10.1017/S1751731108002486>.
- Schiano, A. N., W. S. Harwood, and M. A. Drake. 2017. A 100-Year Review: Sensory analysis of milk. *J. Dairy Sci.* 100:9966–9986. <https://doi.org/10.3168/jds.2017-13031>.
- Schwab, C. G., and G. A. Broderick. 2017. A 100-Year Review: Protein and amino acid nutrition in dairy cows. *J. Dairy Sci.* 100:10094–10112. <https://doi.org/10.3168/jds.2017-13320>.
- Seguel, G., J. P. Keim, E. Vargas-Bello-Perez, C. Geldsetzer-Mendoza, R. Ibañez, and C. Alvarado-Gilis. 2020. Effect of forage brassicas in dairy cow diets on the fatty acid profile and sensory characteristics of Chanco and ricotta cheeses. *J. Dairy Sci.* 103:228–241. <https://doi.org/10.3168/jds.2019-17167>.
- Seymour, W. M., D. R. Campbell, and Z. B. Johnson. 2005. Relationships between rumen volatile fatty acid concentrations and milk production in dairy cows: A literature study. *Anim. Feed Sci. Technol.* 119:155–169. <https://doi.org/10.1016/j.anifeedsci.2004.10.001>.
- Stefanski, E. P., S. C. Garcia, S. R. Farina, D. K. Y. Tan, and D. Tanner. 2010. Effects of sowing rate and grazing management of forage rape (*Brassica napus*) on grazing behaviour and utilisation by dairy cattle. *Anim. Prod. Sci.* 50:560–567. <https://doi.org/10.1071/AN09206>.
- Sun, X. Z., G. Henderson, F. Cox, G. Molano, S. J. Harrison, D. Luo, P. H. Janssen, and D. Pacheco. 2015. Lambs fed fresh winter forage rape (*Brassica napus* L.) emit less methane than those fed perennial ryegrass (*Lolium perenne* L.), and possible mechanisms behind the difference. *PLoS One* 10:e0119697. <https://doi.org/10.1371/journal.pone.0119697>.
- Sun, X. Z., G. C. Waghorn, S. O. Hoskin, S. J. Harrison, S. Muetzel, and D. Pacheco. 2012. Methane emissions from sheep fed fresh brassicas (*Brassica* spp.) compared to perennial ryegrass (*Lolium perenne*). *Anim. Feed Sci. Technol.* 176:107–116. <https://doi.org/10.1016/j.anifeedsci.2012.07.013>.
- Tavendale, M. H., L. P. Meagher, D. Pacheco, N. Walker, G. T. Attwood, and S. Sivakumaran. 2005. Methane production from

- in vitro rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. *Anim. Feed Sci. Technol.* 123–124:403–419. <https://doi.org/10.1016/j.anifeedsci.2005.04.037>.
- Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci.* 18:104–111. <https://doi.org/10.1111/j.1365-2494.1963.tb00335.x>.
- Totty, V. K., S. L. Greenwood, R. H. Bryant, and G. R. Edwards. 2013. Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. *J. Dairy Sci.* 96:141–149. <https://doi.org/10.3168/jds.2012-5504>.
- Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. *J. Dairy Sci.* 48:1215–1223. [https://doi.org/10.3168/jds.S0022-0302\(65\)88430-2](https://doi.org/10.3168/jds.S0022-0302(65)88430-2).
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
- Wang, C., J. X. Liu, Z. P. Yuan, Y. M. Wu, S. W. Zhai, and H. W. Ye. 2007. Effect of level of metabolizable protein on milk production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 90:2960–2965. <https://doi.org/10.3168/jds.2006-129>.
- Weatherburn, M. 1967. Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.* 39:971–974. <https://doi.org/10.1021/ac60252a045>.
- Westwood, C. T., and H. Mulcock. 2012. Nutritional evaluation of five species of forage brassica. *Proc. N. Z. Grassl. Assoc.* 74:31–38.
- Wiedenhoft, M. H., and B. A. Barton. 1995. Taste quality of milk from dairy-cows fed forage brassica cv Tyfon. *J. Sustain. Agric.* 5:139–146. https://doi.org/10.1300/J064v05n03_11.
- Williams, S. R. O., P. J. Moate, M. H. Deighton, M. C. Hannah, W. J. Wales, and J. L. Jacobs. 2016. Milk production and composition, and methane emissions from dairy cows fed lucerne hay with forage brassica or chicory. *Anim. Prod. Sci.* 56:304–311. <https://doi.org/10.1071/AN15528>.
- Zebeli, Q., J. R. Aschenbach, M. Tafaj, J. Boguhn, B. N. Ametaj, and W. Drochner. 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *J. Dairy Sci.* 95:1041–1056. <https://doi.org/10.3168/jds.2011-4421>.
- Zhao, X. H., C. J. Liu, Y. Liu, C. Y. Li, and J. H. Yao. 2013. Effects of replacing dietary starch with neutral detergent-soluble fibre on ruminal fermentation, microbial synthesis and populations of ruminal cellulolytic bacteria using the rumen simulation technique (RUSITEC). *J. Anim. Physiol. Anim. Nutr. (Berl.)* 97:1161–1169. <https://doi.org/10.1111/jpn.12025>.

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